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FURTHER OBSERVATIONS ON THE STRUCTURE AND FUNCTION OF THE OPERCULUM IN *SPIRORBIS MOERCHI* (SERPULIDAE: SPIRORBINAE)

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An important aspect of the reproductive biology of the ubiquitous Spirorbinae is the fact that all known species brood their embryos until larval release. Brood protection occurs either within the parental tube or within a modified operculum, and the mode of brood protection has been proposed as a basis for taxonomic reclassification (Bailey, 1969). Of the two types of brood protection, incubation within the operculum is the most specialized and has been considered a recent development in the evolution of the subfamily (Elsler, 1907; Borg, 1917; Gravier, 1923). Just how newly spawned oocytes enter the opercular brood chamber remains controversial. Oocytes have been described as traveling through the thoracic coelom and subsequently being squeezed into the brood chamber *via* the opercular peduncle (Vuillemin, quoted by Knight-Jones and Knight-Jones, 1974). Such a mechanism would be impossible in *Spirorbis moerchi* because the opercular peduncle is solid and the only entrance into the brood chamber is by means of a pore in the opercular epithelium (Potswald, 1968). The opercular pore in *S. moerchi* has been observed to open wide enough to allow the release of larvae. It can be argued that opening of the opercular pore during larval release is simply a passive phenomenon resulting from force applied by the moving larvae; however, in most cases, the operculum receives another brood shortly after larval release and the pore is then observed to be tightly closed, suggesting that closing of the pore is an active process. It can be further argued that opening, like closing, of the pore may be under active control because the pore appears closed in a virgin operculum prior to deposition of the first brood (Potswald, 1968).

There have been recent studies on opercular histology in several species of *Spirorbis* (Thorp, 1975; Thorp and Segrove, 1975), including one electron microscopic investigation (Bubel, 1973). The latter study, however, fails to give any detailed information concerning the epithelium surrounding the pore. It is the purpose of the present report to provide ultrastructural observations on the opercular epithelium of *S. moerchi*, with particular attention being paid to the epithelium of the pore region in brooding and nonbrooding individuals, in an attempt to correlate morphological detail with function. The results to be presented are an extension of those reported in an earlier study of opercular histology at the resolution of light microscopy (Potswald, 1968).

Materials and Methods

Adult specimens of *Spirorbis moerchi* were collected in Argyle creek on San Juan Island, state of Washington. Worms were removed from their calcareous

tubes by means of heavy dissecting needles. Opercular brood pouches containing embryos, at various stages of development, together with pouches from which larvae recently emerged, were removed at the base of the opercular crown with #5 watchmaker's forceps. The brood pouches were fixed in an ice-cooled mixture of one part 5% glutaraldehyde, one part 5% osmium tetroxide, and two parts of 0.2 M phosphate buffer at pH 7.4 (Stanley, 1967). Numerous attempts to use glutaraldehyde, utilizing a number of different buffers and made isotonic with sea water, as a primary fixative have not been successful with this material. Following fixation, the opercular brood pouches were dehydrated in a graded series of alcohols from 30% to absolute and then embedded in Epon 812 (Luft, 1961). Brood pouches were flat embedded, cut out of block, and oriented, in three planes, on chucks. Each specimen was pared down with a razor blade to just within the region of the pore. One micron thick sections were then cut with a Porter Blum MT-1, affixed to glass slides and stained with Richardson's stain (Richardson, Jarett, and Finke, 1960). Adjacent thin sections were cut with a diamond knife, picked up on 200 mesh grids, and stained in saturated 50% alcoholic uranyl acetate for 20 minutes followed by lead citrate reagent (Reynolds, 1963) for five minutes. The specimens were examined and photographed with an RCA EMU-3E electron microscope.

Results

The operculum of *Spirorbis moerchi* contains a spacious cavity, the brood chamber, and is capped by a characteristically bilobed calcareous plate (Fig. 1). As observed from the side with a dissecting microscope, a thickening, indicating the site of the opercular pore, can be seen in the opercular epithelium. A section through a brood pouch containing embryos shows that the epithelium surrounding the opercular pore is simple columnar, the cells varying in height from about 20 to 25 microns (Fig. 2). A distinct cuticle can be seen to cover the epithelium and during brooding the cuticle tends to almost completely occlude the opercular opening. Light microscopy fails to provide evidence for the presence of muscle in the area surrounding the opercular pore.

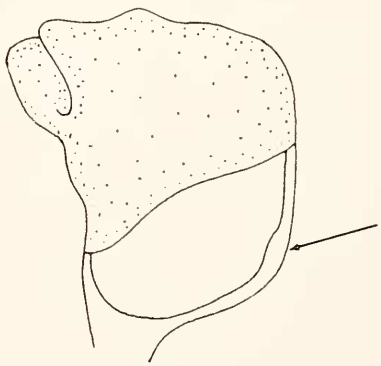
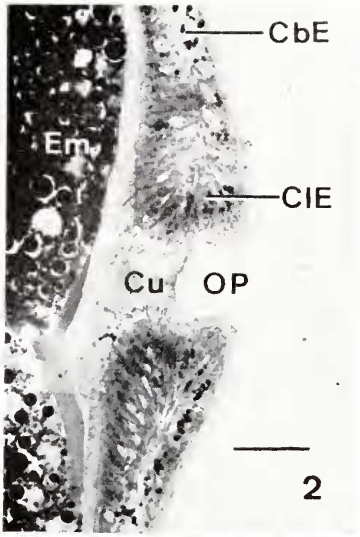


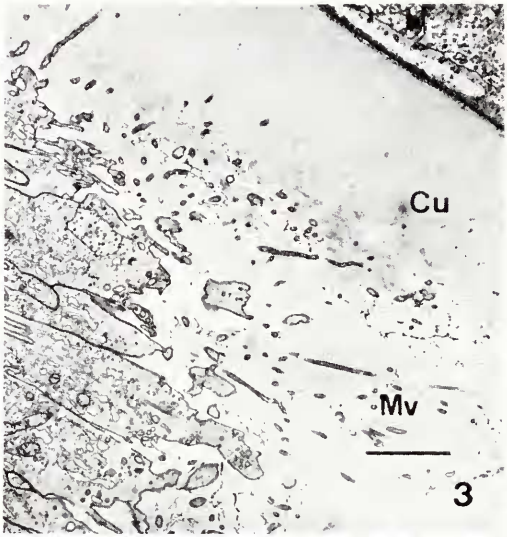
Figure 1. Drawing of a lateral view of a brooding operculum of *S. moerchi* showing the bilobed calcareous plate (stippled area), and the thickened region of the brood pouch epithelium indicating the site of the opercular pore (arrow). Embryos not shown.

An examination of the pore epithelium with the electron microscope during brooding reveals that the epithelial cells possess a narrow diameter from base to apex (Figs. 3, 4). Long irregular microvilli extend from the apex of the cells into the cuticle which nearly occludes the pore (Fig. 3). The cells are joined together by subapical septate junctions arranged in zonulae; however, at the apex, the cells are separated by a relatively wide intercellular space which extends for a considerable length (Figs. 3, 5, 6). In addition to septate junctions, the lateral surfaces of the cells exhibit simple interdigitations (Figs. 3, 4). A distinct basal lamina is absent and the inner and outer epithelium of the pore is separated by an intercellular space containing reticular-like fibers (Fig. 4). Elongate nuclei are basally situated and each contains a nucleolus and scattered chromatin. Dense granules, probably representing pigment responsible for the orange coloration of the operculum in life, are found throughout the cytoplasm (Fig. 4). Portions of the dense granules leach out during fixation, suggesting that the granules contain lipid, perhaps associated with the suspected carotenoid. Each cell contains a well-developed Golgi complex apical to the nucleus together with multivesicular bodies and numerous individual vesicles (Fig. 5). Besides the afore-mentioned organelles and inclusions, the cytoplasm contains mitochondria of conventional morphology, free ribosomes, and relatively little endoplasmic reticulum.

Aside from shape, the most interesting aspect of the cells surrounding the pore during brooding is the presence of numerous microfilaments. The microfilaments, 50–70 Å in diameter, are found individually throughout the cytoplasm and form a dense, felt-like, circumferential network adjacent to the apical plasmalemma. Microfilaments are also seen forming bundles which run parallel to the axis of the cell and extend from the base apically into the microvilli (Figs. 4, 5, 6). The bundles of microfilaments do not appear to attach basally.

The columnar epithelium surrounding the opercular pore is continuous with the cuboidal epithelium which comprises the rest of the noncalcified portion of the opercular brood pouch. The outer cuboidal epithelial layer is about 6 microns thick; whereas, the inner epithelial layer is somewhat thinner (Fig. 7). As in the region of the pore, the inner and outer epithelial layers are separated by an intercellular space containing reticular-like fibers. Unlike the region of the pore, a distinct basal lamina can be observed underlying inner and outer epithelia. Both inner and outer epithelial layers have apical microvilli which penetrate the cuticle; however, the microvilli are much shorter and more regular in appearance than in the pore region. Dense granules, presumably pigment, and intracellular vacuoles are present in both inner and outer epithelia, but are more prevalent in the latter. The vacuoles tend to be much smaller in brooding than in nonbrooding opercula, and, in fact, are difficult to observe in brooding opercula at the light microscopic level (Potswald, 1968). Bundles of microfilaments are present in both epithelial layers, but, contrary to the bundles observed in the columnar epithelium surrounding the pore, these definitely attach to basal hemidesmosomes (Fig. 7, inset). Other than the above-mentioned differences, the cytoplasmic organelles are similar to those observed in the pore epithelium. One further point of interest is the fact that the cells comprising the inner and outer cuboidal epithelia are closely apposed apically and do not exhibit the intercellular spaces observed separating the apical portions of the columnar cells surrounding the pore of a brooding operculum.





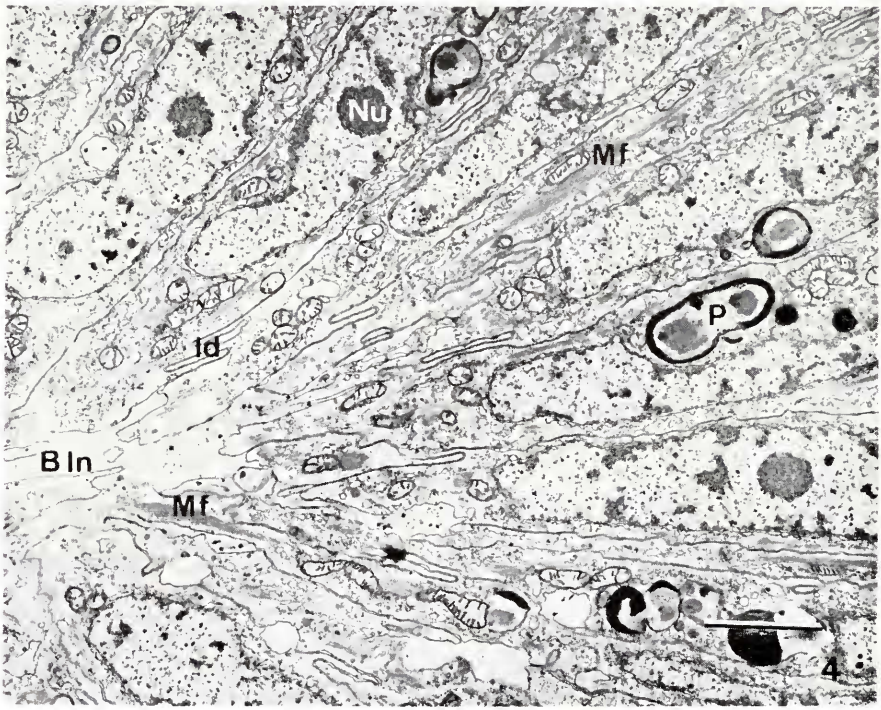


FIGURE 2. Light micrograph of a sagittal section through a brooding operculum showing the columnar epithelium (ClE) bordering the opercular pore (OP), cuboidal epithelium (CbE), cuticle (Cu), and embryos (Em) within the brood chamber. Scale equals 20 μ.

FIGURE 3. A thin section through the apical region of the columnar epithelium bordering the pore of a brooding operculum. Note the narrow diameter of the epithelial cells, the long microvilli (Mv) penetrating the filamentous cuticle (Cu), and a portion of an embryo (Em) within the brood chamber. Scale equals 2 μ.

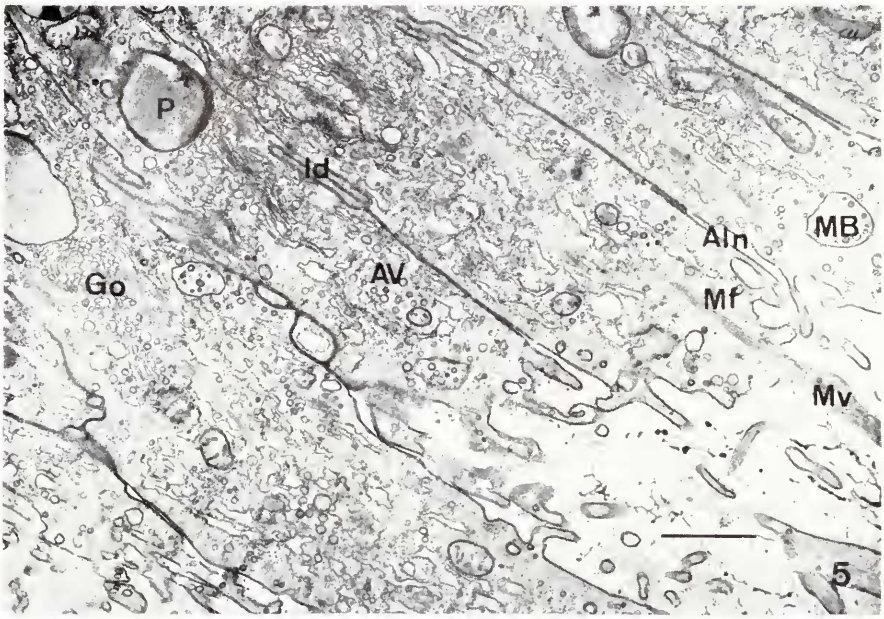
In an attempt to observe changes in the columnar epithelium when the opercular pore is wide open, brood pouches were fixed after the emergence of larvae. Ideally, one would like to observe changes in the epithelium immediately after the release of larvae. Unfortunately, larval release has been observed fortuitously only on a few occasions (Potswald, 1968); consequently, the following observations are based on opercula fixed within 12 hours of larval release and prior to the deposition of a new brood. In each of the three cases to be reported on, the opercular pore, as determined by examination with a dissecting microscope, was open but not maximally, *i.e.*, not wide enough to allow the simultaneous release of two larvae at a time. Further difficulties were encountered upon fixation when the opercular pores tended to close still further upon contact with the fixative. Nevertheless, some interesting differences have been observed in the pore epithelium of nonbrooding opercula.

Upon widening of the brood pore during larval release, one would predict that the previously columnar epithelial cells closing the pore would change shape and take on the characteristics of a cuboidal or even squamous epithelium. This prediction seems to be realized even in opercula whose pores are only perhaps half open (Fig. 8). The cells are reduced in height and consequently exhibit a greater diameter and concomitant reduction in length of microvilli as compared with the columnar cells of brooding opercula. With a shortening of the cells there is a corresponding decrease in the length and width of the intercellular spaces seen separating the columnar cells apically in the brooding condition. Since the empty brood pouches observed here did not have maximally opened pores, one might expect to see cells intermediate in shape between the tall columnar cells of the brooding operculum and the shortened condition of the epithelium surrounding the partially opened pore of the nonbrooding operculum. Such cells have been observed, but they are not nearly as columnar as those surrounding the pore of a brooding operculum (Fig. 8). Bundles of microfilaments running parallel to the cell axis, as observed in the columnar cells of the brooding operculum, are absent in the cells surrounding the opening of the nonbrooding operculum. Instead, the microfilaments form a rather dense mat just below the flattened apical plasma membrane (Fig. 8). In lateral view, the feltwork of microfilaments can be seen to exclude membranous organelles from the apical region. *En face* views of sections taken parallel to and just below the free surface of the epithelium show microfilaments coursing near the lateral cell membranes (Fig. 9). A dense region closely applied to the cytoplasmic surface of the cell membrane suggests an intimate association between microfilaments and the lateral plasmalemma. Although the extensive filamentous network is absent, it will be recalled that the circular dense band of membrane associated microfilaments is present apically in the columnar cells of the brooding operculum.

Discussion

Although the epithelium comprising the opercular wall in *Spirorbis moerchi* is a double layer, it is, nevertheless, a simple epithelium. Both the inner and the

Figure 4. Basal region of the columnar epithelium of a brooding operculum showing elongate nuclei, each of which contains a single nucleolus (Nu), dense granules believed to be pigment (P), lateral interdigitations (Id), basal intercellular space (BIn), and longitudinal bundles of microfilaments (Mf). Scale equals 2 μ.



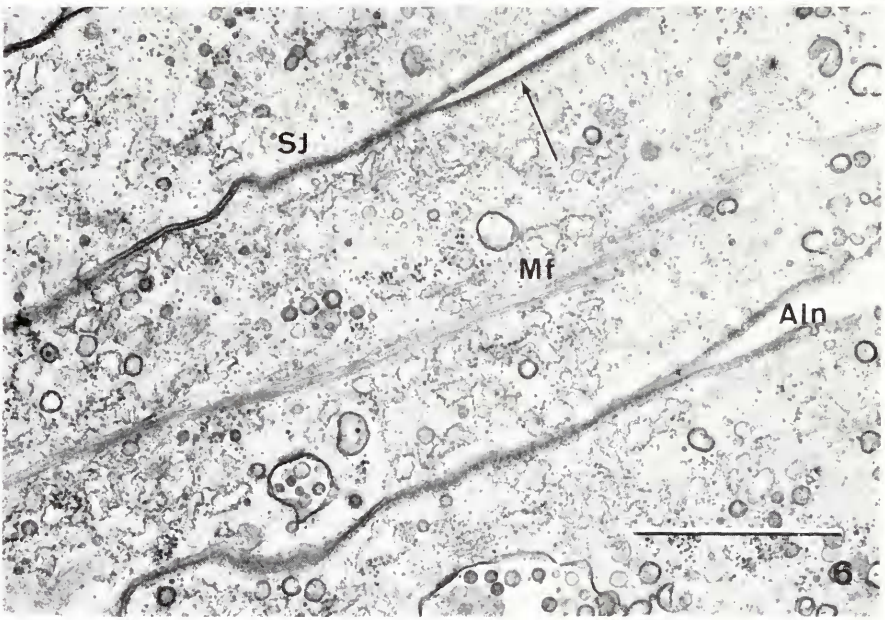


FIGURE 5. Apical region of the pore epithelium of a brooding operculum showing that each cell has an apical Golgi complex (Go), multivesicular bodies (MB), pigment granules (P), lateral interdigitations (Id), apical vesicles (AV), microvilli (Mv), and longitudinal bundles of microfilaments (Mf). Note also that the epithelial cells are separated by apical intercellular spaces (AIn). Scale equals 1 μ.

FIGURE 6. Similar to Figure 5 but at higher magnification, showing septate junctions (SJ), apical intercellular spaces (AIn), longitudinal bundles of microfilaments (Mf), and circumferential network of microfilaments adjacent to the apical plasmalemma (arrow). Scale equals 1 μ.

outer epithelium has its own basal lamina; sandwiched between, and separating the latter, is a reticular lamina. As the two layers converge upon the opercular pore, a basal lamina is difficult to demonstrate and the cuboidal epithelial layers become confluent with the columnar epithelium bordering the pore. How the double nature of the opercular epithelium arises has been explained by Thorp (1975). According to Thorp, during development, the opercular ampulla is composed of a single epithelial layer. As development proceeds, the ampullar epithelium involutes, leaving the opercular pore at the site of involution. This would account not only for the double nature of the opercular epithelium, but also for the presence of an inner cuticle. It also helps explain why the calcareous plate in *S. moerchi* forms inside the brood chamber between the inner epithelium and inner cuticle (Potswald, 1968). A fine structural study of opercular development would be of interest to determine whether, as might be expected, microfilaments play a role in the process of ampullar involution.

The epithelium making up the epidermis of the annelidan body wall has been described as containing basal cells, specialized gland cells, and exhibiting a pseudostratified appearance (Coggeshall, 1966; Potswald, 1971; Burke, 1974). Although the latter features are not found in the operculum, the septate junction in the form of a belt encircling the cell, apical microvilli, and cuticle are characteristics common to both body wall epidermis and the opercular epithelium. The filamentous cuticle of the brood pouch is similar to the epidermal cuticle described in a few species of polychaetes by Storch and Welsch (1970), and it in no way differs from the rest of the cuticle covering the body of *S. moerchi* (Potswald, unpublished observation). Bubel's (1973) description of the opercular cuticle in *S. borealis* is similar to that given here for *S. moerchi;* however, he reports that the opercular cuticle in *S. granulatus* and *S. pusilloides* is much more complex and contains distinctly ordered fibers. It would appear, as pointed out by Storch and Welsch (1970), that there is no correlation between the degree of cuticular complexity and the type of environment inhabited by polychaetes. There is still much to be learned concerning the formation and maintenance of the cuticle in polychaetes; however, it is likely that the well-developed apical Golgi complex found in both cuboidal and columnar cells of the opercular epithelium may play an important role, a suggestion also offered by Bubel (1973).

Dense granules present in the epithelial cells of the brood pouch, which are interpreted to be pigment granules imparting the orange color to the operculum in life, are similar to the dense granules observed in the epidermal cells of *Lumbricus* (Coggeshall, 1966) and *Eisenia* (Burke, 1974). Coggeshall suggests that the granules in *Lumbricus* may be either melanin or lipofuscin in nature. Burke points out that they may be lysosomal and cites evidence for the lysosomal nature of liposfuscin granules in other tissues. In addition to possible lysosomes, Bubel (1973) describes electron dense inclusion bodies in the opercular epithelium of *S. borealis* which he believes may represent a type of secretory product. Information presented in this report on the dense granules in *S. moerchi* does little to settle the question, but the granules appear to be the best candidates to explain coloration of the living epithelium.

A rather interesting aspect of the fine structure of the opercular epithelium is the presence of intracellular vacuoles. In *S. moerchi,* the vacuoles predominate in the cuboidal epithelia of the brood chamber, but they are apparently not unique

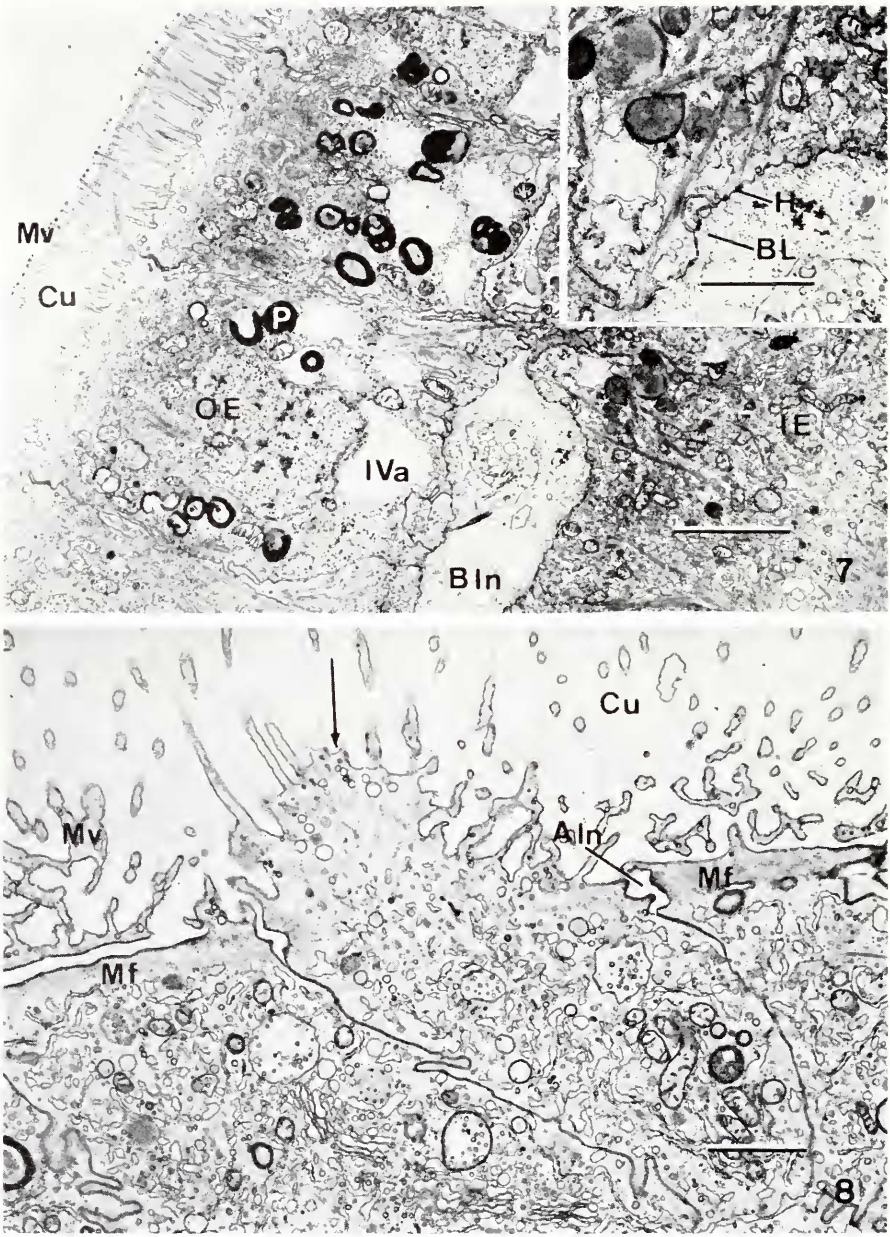


FIGURE 7. A section through the inner (IE) and outer (OE) cuboidal epithelial layers adjacent to the columnar pore epithelium of a brooding operculum showing outer cuticle (Cu), microvilli (Mv), pigment granules (P), and basal intercellular space (BIn). Note that the intracellular vacuoles (IVa) are larger and more abundant in the outer as compared with the inner epithelium. Scale equals 2 μ. The inset (upper right) shows the outer basal lamina (BL) and bundles of filaments attaching to hemidesmosomes (H). Scale equals 1 μ.

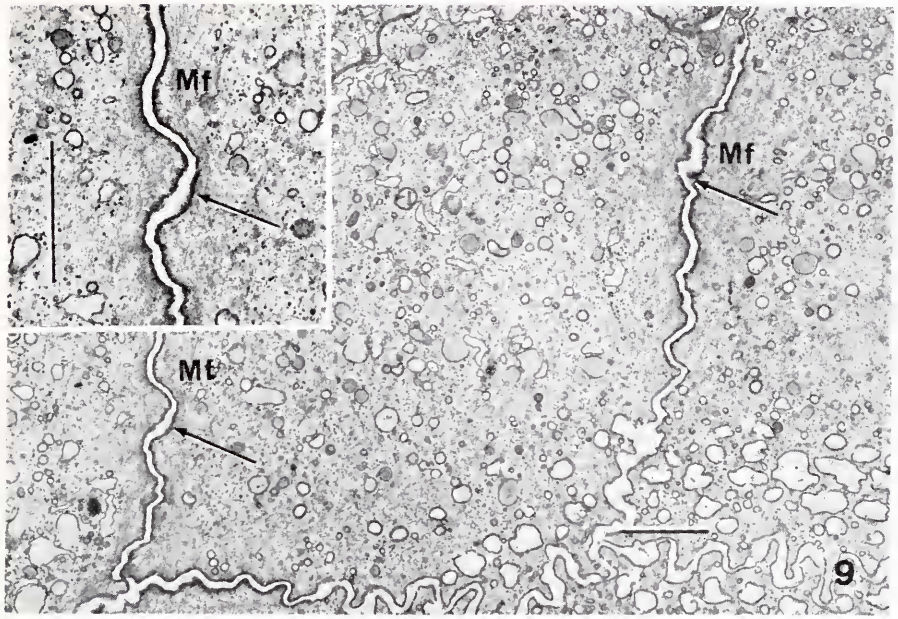


FIGURE 9. *En face* subapical view of the pore epithelium of a nonbrooding operculum showing microfilaments (Mf) coursing near the lateral cell membrane and dense region adjacent to the membrane (arrows). Scale equals 1 μ. Inset (upper left) is an enlargement of the lower left portion of Figure 9. Scale equals 1 μ.

to the epithelial cells of the brood chamber. After examining the epidermis in 18 polychaete species, Storch and Welsch (1970) describe the presence of numerous intracellular vacuoles in four species (*Autolytus pictus, Ophiodromus flexuosus, Branchiomma bombyx, Myxicola infundibulum*) and report that the largest part of the cytoplasm of the epidermal cells of *Chaetopterus variopedatus* is occupied by one large vacuole. The latter investigators could find no evidence that the vacuolated cells were restricted to certain systematic groups or species inhabiting a particular environment. Be this as it may, the very presence of the intracellular vacuoles would seem to suggest that they must have some function. Bubel (1973) has suggested that the vacuoles may play a role in cuticle and/or basal lamina secretion. The latter hypothesis is quite plausible and could be tested with high resolution autoradiography. At the light microscopic level, the vacuoles in *S. moerchi* are more apparent in nonbrooding opercula than in brooding opercula (Potswald, 1968). Such an observation might be taken to indicate that the intracellular vacuoles may function to allow the epithelium to expand upon reception of a brood. If the vacuoles are to collapse and thereby cause an expansion of the opercular epithelium, the epithelium must have a mechanism by means of which the contents of the vacuoles are expelled. The pinocytotic

FIGURE 8. Apical region of the pore epithelium of a nonbrooding operculum showing the filamentous cuticle (Cu), microvilli (Mv), decreased apical intercellular space (AIn), and dense mat of microfilaments (Mf) below the flattened apical plasma membrane. Note that one of the cells appears to be elongating (arrow). Scale equals 1 μ.

vesicles observed in the apical ends of the opercular epithelial cells could possibly serve to accomplish this feat. It is just as reasonable to suggest, however, that the pinocytotic vesicles are of Golgi origin and are maintaining the cuticle.

Evidence has previously been presented (see Introduction) which supports the contention that the opercular pore in *S. moerchi* is capable of actively closing and perhaps opening. Light microscopy has revealed the presence of longitudinal muscle fibers embedded within the connective tissue of the opercular peduncle (Potswald, 1968; Thorp and Segrove, 1975); however, the present study has conclusively shown that muscle fibers do not penetrate between the two epithelial layers making up the opercular brood chamber. Since the important study of Cloney (1966) on the contractile role of the caudal epithelium during tail resorption in ascidian larvae, numerous reports have documented the close correlation between contraction and the presence of cytoplasmic microfilaments in a variety of nonmuscle cells (Baker and Schroeder, 1967; Schroeder, 1969, 1970, 1972, 1973; Spooner, Yamada, and Wessells, 1971; Wessells, Spooner, Ash, Bradley, Luduena, Taylor, Wrenn, and Yamada, 1971; Conrad, 1973; just to cite a few). That cytoplasmic microfilaments, as observed in fixed and embedded tissue cells, are real and not artifacts of preparation has been convincingly argued by Buckley (1975). Although experimental evidence is presently lacking, it seems reasonable to suggest that the presence of microfilaments in the columnar cells bordering the opercular pore in *S. moerchi* are responsible for the cellular shape changes observed in brooding and nonbrooding opercula.

In the tall columnar cells bordering the closed pore of a brooding operculum, microfilaments are found individually throughout the cytoplasm and form a circumferential, dense, felt-like network adjacent to the apical plasmalemma. The latter may correspond to the subplasmalemmal filament network described by Buckley (1975) in cultured cells. In addition to the network of microfilaments, longitudinal bundles of microfilaments are also observed within the columnar cells bordering the pore and, unlike bundles of filaments seen in the cuboidal epithelium of the operculum apparently do not attach to hemidesmosomes. Whether these longitudinal bundles correspond to the stress fibers observed in tissue culture cells (Buckley, 1975) is not clear. In nonbrooding opercula with partially opened pores, the cells bordering the opening show decreased height and a shortening in length of apical intercellular spaces. Microfilaments form a network under the apical plasma membranes of the flattened cells, and, subapically, a ring of microfilaments closely applied to the lateral plasmalemma can be seen. Bundles of microfilaments arranged parallel to the longitudinal axis, as seen in the brooding condition, are absent. Investigators of nonmuscle cells capable of contraction (see references cited earlier) when considering the role of microfilaments in causing cell constriction, which in the case of *S. moerchi* would lead to closure of the opercular pore, all propose that rings of microfilaments are mechanically responsible for the contractile event. Observations on opercular pore function in *S. moerchi* are consistent with the afore-mentioned hypothesis. For reasons that have already been explained, a completely "open" or "relaxed" pore condition has not been observed in *S. moerchi;* consequently, the description given for the pore epithelium in the nonbrooding operculum is a description for an epithelium in the process of contracting. As apical contraction proceeds, the cells elongate and close the pore. The apical ring of membrane associated microfilaments is retained in the brooding condition and is believed to maintain the columnar shape of the epithelial cells. During

larval release, widening of the pore may not be due entirely to changes in the columnar epithelium bordering the pore. Whereas the columnar epithelium approaches the cuboidal or even squamous condition, the cuboidal epithelium in the noncalcified region of the operculum may also change shape and become more columnar. The latter possibility should be explored.

Just what controls opening and closing of the opercular pore is not known. Larval release *via* the pore has been observed in *S. moerchi* (Potswald, 1968), and it is certain that the pore remains open wide enough to accept another brood. Opening may be a passive phenomenon due to the movement of the larvae within the brood chamber but after receiving a new brood, the pore closes and this would seem to be an active process. Perhaps when newly spawned oocytes are packed into the brood chamber, recently vacated by larvae, the opercular epithelium stretches, and this stretching somehow triggers contraction of the pore epithelium. More difficult to explain, however, is how oocytes are transferred into a virgin operculum in which the pore, at least by examination with a dissecting microscope, appears to be closed (Potswald, 1968). Thorp and Segrove (1975) have described the oocytes of *S. spirorbis* as being very fluid during spawning and capable of flowing through an opening with a diameter one-tenth or less that of the oocyte itself. If the oocytes of *S. moerchi* are equally malleable, and they may well be since it is believed that they squeeze through the body wall during spawning (Potswald, 1967), then perhaps it is not too difficult to understand how they gain entrance into a virgin operculum, given that the pore is at least partially patent. However, if the pore of a virgin operculum does in fact actively open to receive the first brood, one would have to postulate the existence of some sort of stimulus which would cause a relaxation of the apical ring of microfilaments and a concomitant contraction of the epithelial cells in an apical to basal direction. The bundles of microfilaments observed parallel to the long axes of the columnar cells in the brooding condition, assuming they are also present in the virgin operculum, could possibly play a role in the hypothetical event. Unfortunately, as Thorp and Segrove (1975) point out, neither they nor any other worker have ever observed the actual transfer of oocytes into an opercular brood chamber.

I wish to thank Dr. Robert L. Fernald, retired Director of the Friday Harbor Laboratories, for providing excellent facilities at the laboratories during the time that the material for this report was collected.

Summary

1. The ultrastructure of the opercular epithelium in *Spirorbis moerchi,* with special emphasis on the structure of the pore epithelium in brooding and nonbrooding individuals, has been described.

2. In brooding worms, the epithelium bordering the closed opercular pore consists of tall columnar cells. Each cell is characterized by the presence of long microvilli, apical intercellular spaces, an apical ring of membrane associated microfilaments, and bundles of microfilaments arranged parallel to the longitudinal axis.

3. After larval release, the opercular pore remains open, although not maximally, and the cells bordering the pore, including their microvilli and apical intercellular spaces, are now reduced in height. The cells contain an apical filamentous network and a subapical ring of membrane associated microfilaments, but lack the longitudinal bundles observed in the brooding condition.

4. The role of microfilaments, together with other aspects of the ultrastructure of the opercular epithelium, are discussed.

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